

CHAPTER 3

INVESTIGATING LIGHT PROPAGATION IN FULL AND SKIMMED MILK BASED ON SPECTROSCOPY AND MONTE CARLO ANALYSIS

This chapter analyses both experimental and theoretical analysis on full milk and skimmed milk based on milk fat content. The light experiments are carried out based on Visible (VIS), Near InfraRed (NIR) and Infra-Red (IR) spectra whereas Monte Carlo theory is used to analyse the effect of various internal reflections, the photon count and loss for both skimmed milk and full milk.

This chapter is done to achieve the first objective; to investigate and compare light propagation in milk using various spectrometers.

Results in Chapter 3 have been published as:

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3.1 Introduction

Milk quality can be determined through its fat composition. It is important to know the fat composition in milk to ensure consumption of the right product for health reasons. Spectrometry can be used to study the fat composition in milk. In this chapter, light propagation in milk based on visible and near infrared (NIR) spectra is investigated. Samples comprise skimmed and full milk. Full milk shows higher absorbance at visible (VIS) spectra compared to skimmed milk. The analysis on NIR spectra also shows that full milk has higher absorbance peak than skimmed milk due to higher amount of fat globule. Both milk samples show higher water element than carbon. Numerical modeling based on Monte Carlo method is also done to support experimental results. The modeling results show that full milk has a larger photon count compared to skimmed milk. This is attributed to the large fat globule in full milk that has higher absorbance over skimmed milk. Thus, characterization of milk fat based on spectroscopy techniques can monitor milk adulteration issues, which indirectly gives us guidance on healthy dairy intakes.

3.2 Research Methodology

The research methodology of this chapter covers both experimental and theoretical approaches. Figure 3.1 shows the fundamental steps taken to complete the study. Fundamentally, this study was initiated by performing research on light propagation. Then, the experimental set up was done using three types of spectrometers that covers visible (VIS), Near Infra-Red (NIR) and Infra-Red (IR) spectra. The experiment was conducted and further analysed by simulating the Monte Carlo theory for milk using Matlab software.

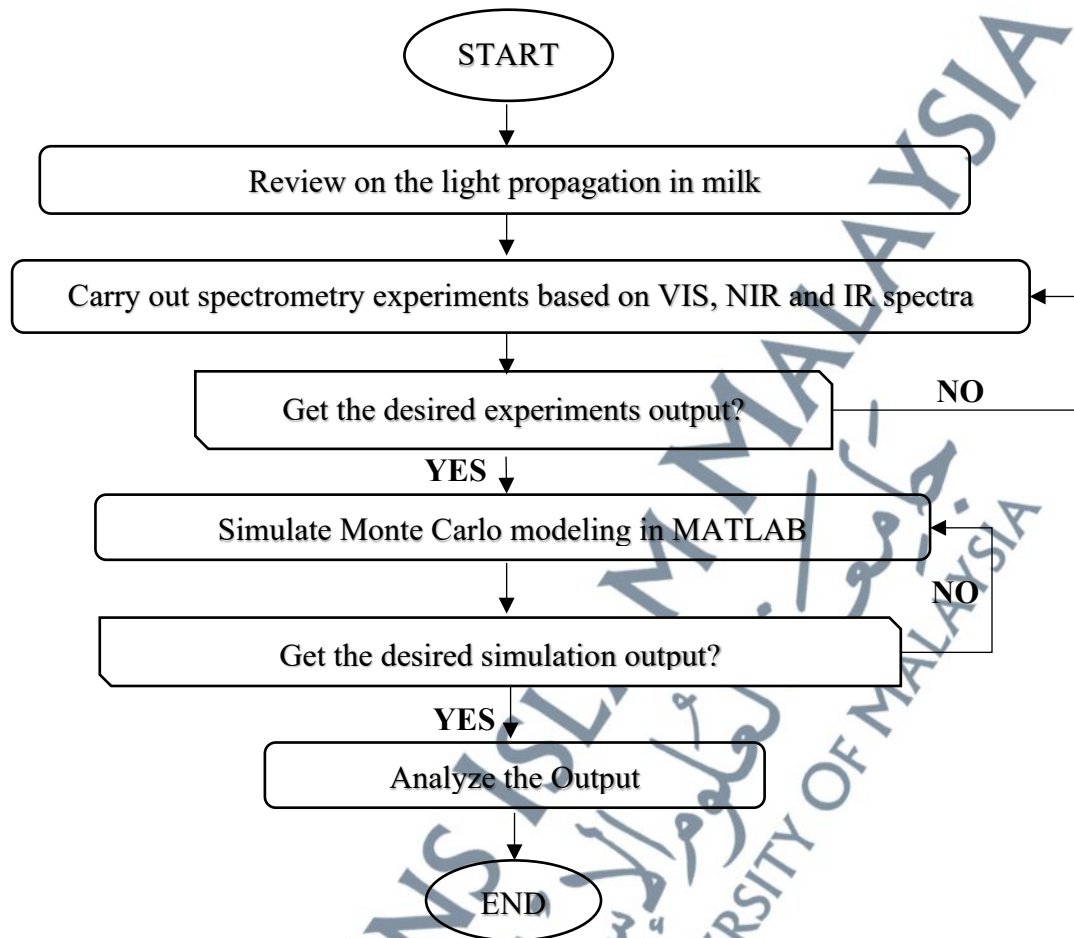


Figure 3.1: Flow Chart of The Milk Fat Analysis.

3.2.1 Materials and experimental set up.

Three different types of spectrometers were used such as Ocean Optic Flame Near-Infrared (NIR) Spectrometer, Perkin Elmer Lambda 750 UV/VIS/NIR Spectrophotometer and Varian 3100 Excalibur Series Fourier Transform Infra-Red (FTIR) Spectroscopy, shown in the figure 3.2, 3.3 and 3.4 respectively. The spectrometers are differentiated based on their ability to capture the light by respective wavelength. The spectrometers used in the experiment can offer large size, cost-effective and reliable technology compared to other methods which measured the fat

content in milk (Amr et al., 2018). Each experiment was repeated for 5 to 10 times to obtain the best average reading of the results.



Figure 3.2: Ocean Optic Near Infra-Red (NIR) Flame Spectrometer.



Source: (LAMBDA 750 UV/Vis/NIR Spectrophotometer | PerkinElmer, 2019)

Figure 3.3: Perkin Elmer Lambda, 750 UV/VIS/NIR Spectrophotometer.



Figure 3.4: Varian 3100 Excalibur Series Fourier Transform Infra-Red (FTIR) Spectroscopy.

3.2.2 Sample Preparations

Ultra-High Temperature (UHT) milk samples from brand ‘Dutch Lady’ comprise of skimmed and full milk were used. Firstly, both samples were diluted with a ratio of 1:100 for water and milk respectively. Distilled water was applied as a reference and comparison with milk samples. The prepared sample was placed in the 10 mm plastic cuvette and lightly shaken and stirred to mix the solution before being placed in Ocean Optic Flame Near-Infrared (NIR) Spectrometer and Perkin Elmer Lambda 750 UV/VIS/NIR Spectrometer at spectrum ranges of 950-1650 nm and 350-860 nm respectively. The Tungsten Halogen Light Source HL-2000-HP-FHSA is used as the source of light in the experiments. Meanwhile, a droplet of undiluted solution from both milk samples was tested in a Varian 3100 Excalibur Series Fourier Transform Infra-Red (FTIR) Spectroscopy to identify and differentiate any compounds exist in

both samples. The wavelength range for the FTIR Spectroscopy is from 550 to 3950 cm^{-1} and the analysis was done in Varian Resolution Software. All experiments were repeated 5 times to ensure the result consistency and accuracy. Figure 3.5 shows the diluted samples prepared for the spectrometry experiments.

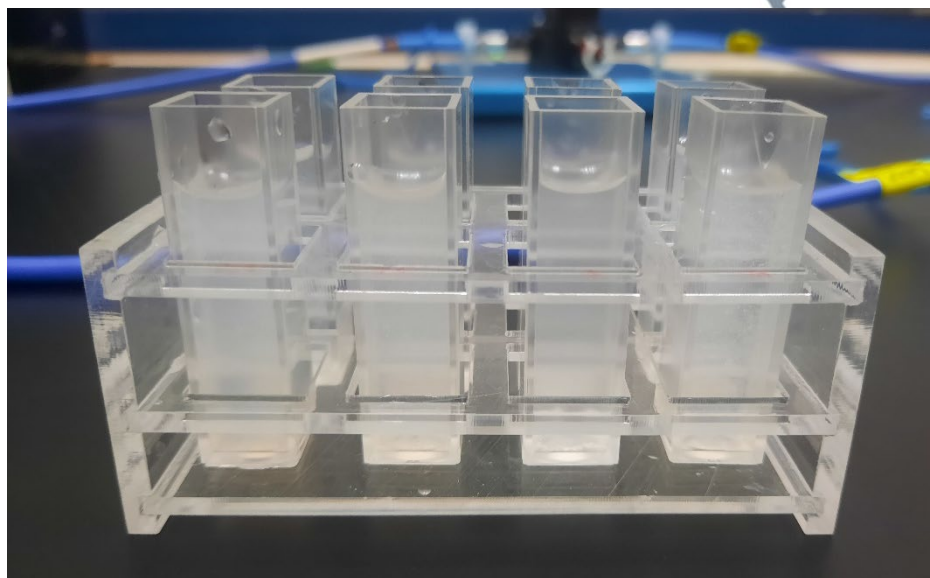


Figure 3.5: The Diluted Samples for Spectrometry Experiments.

3.2.3 Theoretical Analysis

Many modeling techniques can be applied to analyse the light propagation in random media such as Monte Carlo (Vinckenbosch et al., 2015) (Song et al., 2013) (Bocklin, 2014)(Romanov et al., 2012) (Jain & Sarma, 2015) (Forcato et al., 2005), Finite Element Modeling (FEM) (Mahmoud et al., 2012) and Finite Different Time Domain (FDTD) (Botros & Nasr, 2000). Vaskova *et al.* (Vaskova et al., 2016) stated that mathematical evaluation could be used to measure the accurate milk fat content in order to determine the quality control of the product. This research relies on Monte Carlo modeling technique where the photon count and photon loss for milk are simulated. The selected technique was used to distinguish the milk fat between skimmed

and whole milk in which scattering factors such as internal coefficient, anisotropic parameters and forward power were analysed. Besides, Monte Carlo can track the movement of photons inside the turbid medium which experience absorption, scattering and power loss. Fundamentally, the photon propagation was initiated by photon launching followed by photon absorption, scattering, and was terminated by photon detection as depicted in figure 3.6.

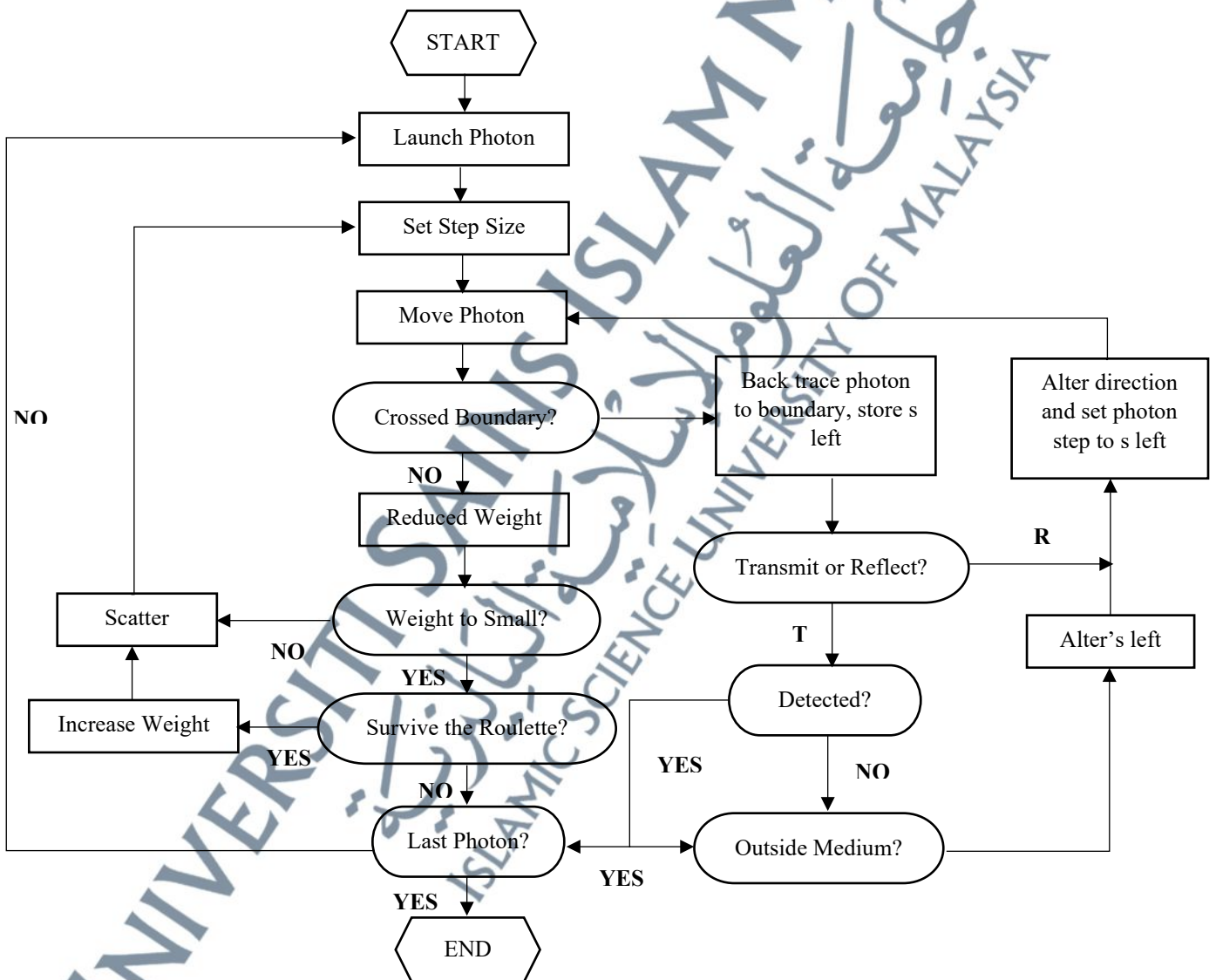


Figure 3.6: Modeling of Light Propagation Based on Monte Carlo Technique.

In Monte Carlo technique, four main steps were used; photon launching, photon absorption, photon scattering and finally, photon detection with continuous propagation (Vinckenbosch et al., 2015).

3.2.4 Photon Initialization

At the first stage, the photon is launched followed by the step length expressed by Equation (3.1) (Song et al., 2013).

$$\Delta s = \frac{-\ln(rnd)}{\mu_t} \quad (3.1)$$

While μ_t refers to internal coefficient, rnd is a random number that is launched between the interval of 0 and 1 and is sampled at every executing loop, μ_a is the absorption coefficient and, μ_s is the scattering coefficient as shown in Equation (3.2) (Bocklin, 2014).

$$\mu_t = \mu_s + \mu_a \quad (3.2)$$

At the same time, virtual weight is assigned to each photon before any movement. Hence, the first photon position is at 3D space, (x_1, y_1, z_1) . The first polar position at 3D space is given by Equation (3.3) (Song et al., 2013).

$$\begin{aligned} x_1 &= x_0 + \mu_x \Delta s \\ y_1 &= y_0 + \mu_y \Delta s \\ z_1 &= z_0 + \mu_z \Delta s \end{aligned} \quad (3.3)$$

3.2.5 Photon Absorption

The second stage involves photon absorption. After the absorption, photon reduces its weight (w) by setting the threshold for every iteration. The reduction of photon weight is given by Equation (3.4) (Bocklin, 2014).

$$w_i = w_{i-1} \left(1 - \frac{\mu_a}{\mu_t}\right) \text{ where } i = 1, 2, 3, \dots \quad (3.4)$$

Russian roulette technique was used in the modeling because the sole calculation time to estimate the light propagation is not realistic. Thus, random value (rnd) is set based on Equation (3.5) (Bocklin, 2014).

$$rnd = \frac{w_0}{10} \quad (3.5)$$

where w_0 refers to the initial photon weight. If the random number exceeds the threshold value, the photon is terminated, photon weight is increased by $1/p$.

3.2.6 Photon Scattering and Propagation

The third stage consists of photon scattering and propagation. When photon reduces its weight, but still alive in the roulette, they can be scattered. Hence, at this stage, the deflection angle can be measured based on the phase function. Deflection angle, θ is calculated based on Equation (3.6) and Equation (3.7) (Bocklin, 2014).

$$\theta = \cos^{-1} \left[\frac{1}{2 \cdot g} \cdot \left\{ 1 + g^2 - \left(\frac{rnd}{H} + (1 + g^{-2\alpha}) \alpha^{-1} \right) \right\} \right] \quad (3.6)$$

$$\text{Where: } H = \frac{(1+g^2)^{2\alpha}}{(1+g)^{2\alpha} - (1-g)^{2\alpha}} \quad (3.7)$$

where g is the anisotropy factor referring to scattering function, H is Henvey-Greenstein phase function and α is the weight factor. The new position (x_1, y_1, z_1) can be generated

using the obtained phase function, deflection angle and the azimuthal angle, ψ . ψ is randomly chosen from the uniform distribution within the interval of $(0, 2\pi)$, expressed by Equation (3.8) to Equation (3.10) (Bocklin, 2014).

$$x_{i+1} = x_i + \Delta s \cdot \left(\frac{\sin(\theta)}{\sqrt{1-u_z^2}} (u_x \cdot u_z \cdot \cos(\varphi) - u_y \cdot \sin(\varphi) u_x \cdot \cos(\theta)) \right) \quad (3.8)$$

$$y_{i+1} = y_i + \Delta s \cdot \left(\frac{\sin(\theta)}{\sqrt{1-u_z^2}} (u_y \cdot u_z \cdot \cos(\varphi) + u_x \cdot \sin(\varphi)) u_y \cdot \cos(\theta) \right) \quad (3.9)$$

$$z_{i+1} = z_i + \Delta s \cdot (-\sin(\theta) \cdot \cos(\varphi) \cdot \sqrt{1-u_z^2} + u_z \cdot \cos(\theta)) \quad (3.10)$$

where, (u_x, u_y, u_z) represents the values of photon propagation at every iteration, i .

3.2.7 Photon Detection

The photon is detected at the final stage. Photon detection is done by considering its weight and the optical properties of the measured medium such as refractive index, number of emitted photons, position, absorption coefficient, α and scattering coefficient (Romanov et al., 2012). Table 3.1 shows the weight of milk fat contents, scattering coefficient and absorption coefficient. The parameters in the table were used to record backscattered count and photon loss during scattering process. The internal coefficient was determined from Equation (3.4) whereas the scattering and absorption coefficients were based on (Qin & Lu, 2007).

Table 3.1: Scattering, Absorption and Internal Coefficients For Both Milk Samples.

Type of medium	Weight of Milk Fat Contents, gram (g)	Scattering coefficient, μ_s	Absorption Coefficient, μ_a	Internal Coefficient, μ_t
Skimmed Milk	0.0	1.65	0.55	2.20
	0.1	1.68	0.56	2.24
Full Milk	3.2	2.36	1.80	4.16
	3.8	2.36	1.9	4.26

Here, the theoretical analysis was used to support the study in terms of light or photon transmitted in milk, not as a direct comparison with the experimental results.

3.3 Results and Discussion

The experiments were done using various spectrometry techniques whereas the modeling was done based on Monte Carlo technique in order to support the experimental study on light propagation in milk.

3.3.1 Experimental Results

The experiment was carried out based on Visible (VIS), Near Infra-Red (NIR) and Infra-Red (IR) spectra.

3.3.1.1 Absorbance Spectra for Visible (VIS) Spectrometer

Figure 3.7 shows the absorbance spectra of skimmed milk, full milk and water at wavelength ranges of 380 nm – 860 nm. The graph was processed in UV WinLab software using Perkin Elmer Lambda, 750 UV/VIS/NIR Spectroscopy. The visible spectra (figure 3.7) were analysed based on spectrum continuity (Jain & Sarma, 2015) instead of observing the milk fat spectrum at certain peaks, as being analysed in Near Infra-Red (NIR) and Infra-Red (IR) spectra (Amr et al., 2018) (Vaskova et al., 2016) (Forcato et al., 2005) (Tsenkova et al., 2000). It is clearly shown that the absorbance of the skimmed milk is much lower than full milk for each wavelength. The almost linear line is observed for water sample due to the weak water absorbance in the visible spectrum than in Near Infra-Red (NIR) and Infra-Red (IR) spectra.

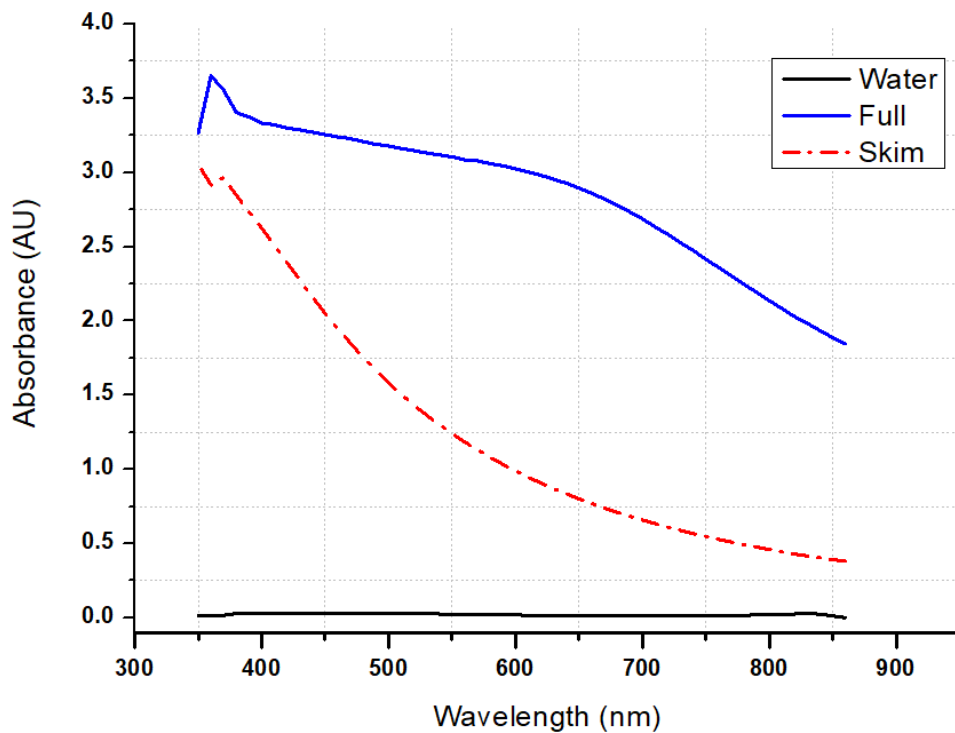


Figure 3.7: Spectrum analysis of Different Milk Samples Versus Wavelength Using Visible (VIS) Spectrometer.

3.3.1.2 Absorbance Spectra for Near Infra-Red (NIR) Spectrometer

Figure 3.8 shows the absorbance spectrum of Near Infra-Red (NIR) for the measured samples of skimmed milk, full milk and water using Ocean Optic NIR Spectrometer at wavelength ranges 960 -1660 nm. From the figure, we observe that the absorbance of full milk shows higher absorbance over skimmed milk and water respectively. The three strong absorption peaks observed at 972, 1158 and 1392 nm in the NIR spectrum. In Figure 3.8, the absorbance shows negative values from 1050 nm until 1100 nm where we attribute that to the background noise and spectrometer calibration.

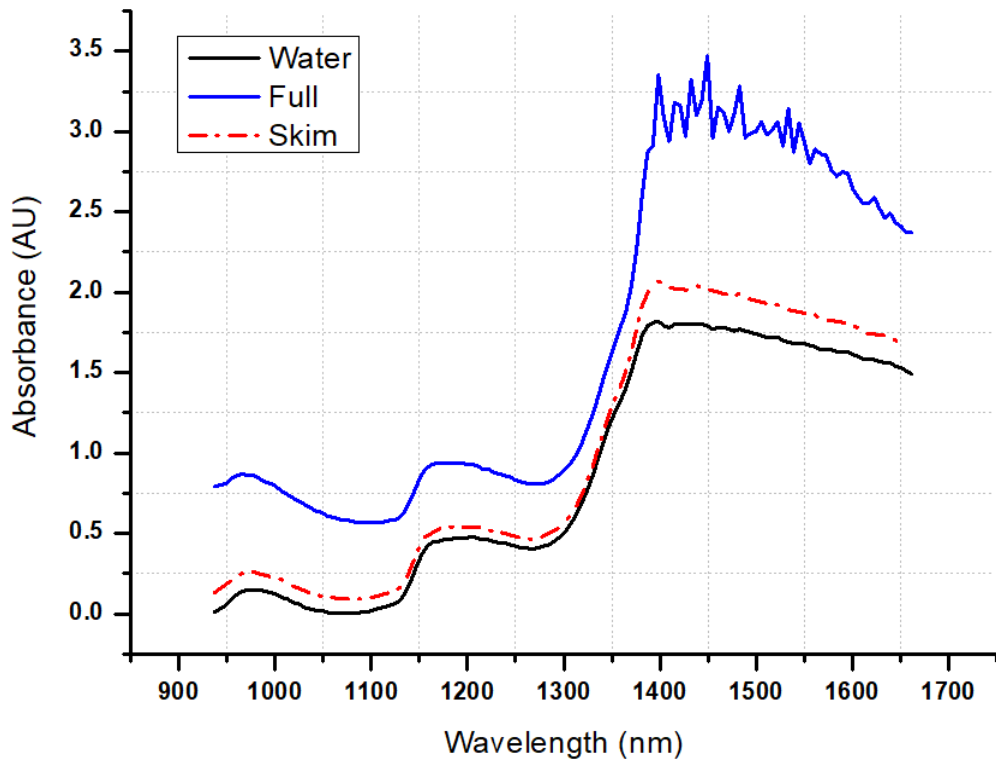


Figure 3.8: Spectrum Analysis of Different Milk Samples For NIR Absorbance Versus Wavelength.

3.3.1.3 Transmission Spectra for Fourier Transform Infra-Red (FTIR) Spectroscopy

Investigating light propagation in milk is continued using Fourier Transform Infra-Red (FTIR) spectroscopy. Transmission spectra for both milk samples are shown in figure 3.9. The figure shows the transmission of two main functional groups in milk samples, which are hydroxyl and carboxyl group. A small valley from 1500-1680 nm represents carboxyl (chemical bond, C=C) and a broad valley from 3300 - 3500 nm represents hydroxyl (chemical bond, O-H) groups. The C, O and H refer to carbon, oxygen and hydrogen respectively. A broad hydroxyl spectrum (O-H) is observed from the graph due to high water composition in the samples. The dominated water bands in

the spectra affect the characteristic absorption of other milk fat components such as triglycerides and saturated fatty acid (Tsenkova et al., 2000). We observe slight changes at carboxyl (C=C) stretch that is attributed to the unsaturated fatty acid which characterizes skimmed and full milk (Amr et al., 2018) (Vaskova et al., 2016) (Tsenkova et al., 2000).

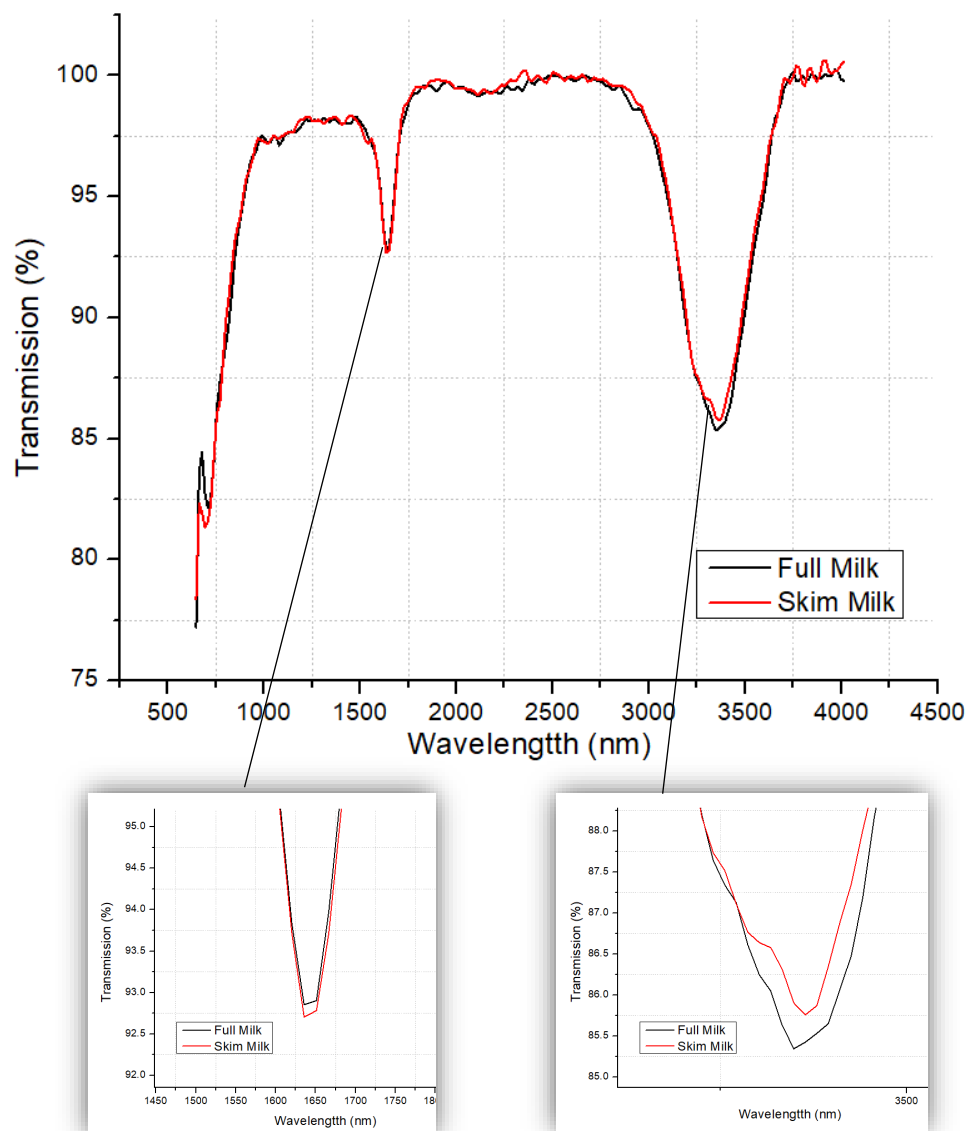


Figure 3.9: Spectrum Analysis of Different Milk Samples Of FTIR Transmittance Versus Wavelength.

In addition, strong water (O-H) absorption was observed at 960, 1440 and 1950 nm using MEMs spectrometer (Amr et al., 2018) whereas Raman spectrometry was used to characterize milk fat consist of triglycerides groups, saturated and unsaturated fatty acid. The characterization is based on chemical bonds C=O, CH₂ and C=C stretching. The functional groups based on absorbance wavelength are summarized in Table 3.2.

Table 3.2: Chemical Compounds at Various Wavelength Ranges In FTIR Analysis.

Wavelength Peak/ Ranges (cm ⁻¹)	Functional Group	Chemical Bonds	Types of Chemical Compounds
3300 – 3500	Hydroxyl	O-H	Water
1500-1680	Carboxyl	C=C	Unsaturated fatty acid

3.3.2 Theoretical Analysis

The Monte Carlo technique was used to measure the forward photon count for different anisotropy, g value in both skimmed and full milk. Henvey-Greenstein suggests three main scattering types based on the anisotropic value, g where purely forward scattering is denoted when $g=1$, while a purely backscattering and isotropic scattering are denoted when $g=-1$ and $g=0$ respectively (S. A. Prahl et al., 1993).

Henvey-Greenstein phase function is commonly used to analyse the light scattering in biomedical fields such as tissues and cell (Vinckenbosch et al., 2015) (Golshan et al., 2011) where most of biological tissues have anisotropic value, g greater than 0.7 ($g>0.7$) (Ding et al., 2009). Hence, forward scattering in the range of $0<g<1$ was applied to analyse the milk fat due to the disordered and random media in both media (Qin & Lu, 2007).

Numerical modeling based on Monte Carlo technique are shown in figure 3.10.

In figure 3.11, different internal coefficients are measured to observe their effects on

various anisotropic parameters. The forward photon count in skimmed milk is measured and compared using internal coefficient of 2.24 and 2.20 respectively (Figure 3.10 (a) and 3.10 (b)). Meanwhile, figure 3.10 (c) and 3.10(d) show the photon count and photon loss of various anisotropy parameters for both samples.

High forward power is analysed when high internal coefficient is applied to the skimmed milk. Besides that, there is a high incline when g parameters approach 1 for both internal coefficients (figure 3.10 (a)). The photon loss of skimmed milk can be analysed by varying the internal coefficient. Based on figure 3.10 (b), when g increases until 0.8, the photon loss increases gradually and starts to decrease when g is larger than 0.8. The measured photon loss for anisotropy parameter, g approaches 1 is lower than the g approaches 0.

Figure 3.10 (c) and (d) distinguish skimmed milk and full milk in terms of photon count and photon loss.

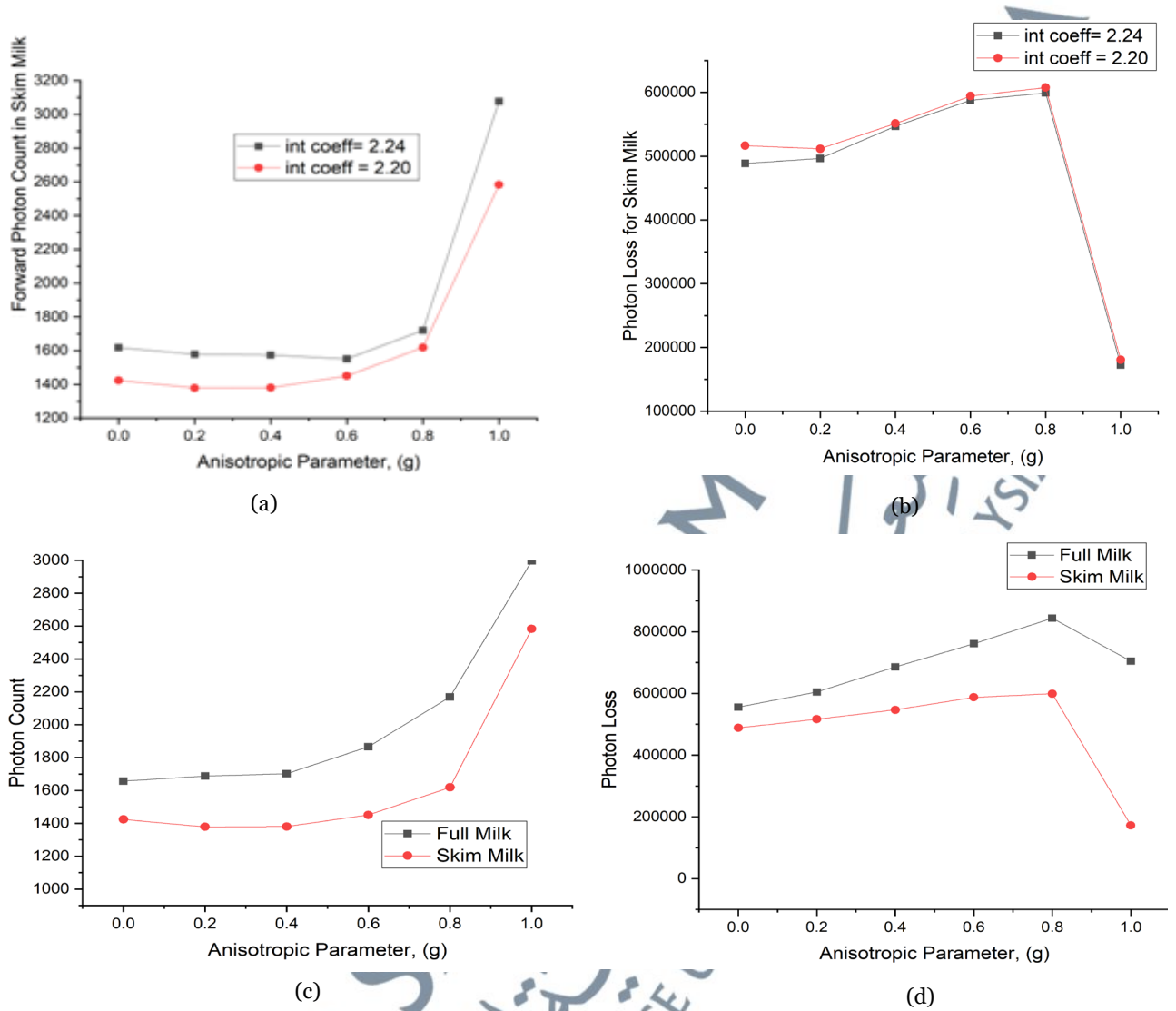


Figure 3.10: (a) Forward Photon Count Of Skimmed Milk (b) Photon Loss With Various Internal Coefficients Measured In Skimmed Milk (c) Photon Count For Skimmed Milk and Full Milk (d) Photon Loss For Skimmed Milk And Full Milk.

Based on the results, forward photon count in full milk is higher than skimmed milk whereas the photon loss in skimmed milk is lower than in full milk. This is due to the fact that skimmed milk has less fat globules than full milk. The large fat molecules in full milk absorb and scatter more light over skimmed milk. Stocker *et al.* (Stocker *et al.*, 2017) have done simulation using scattering coefficient, μ_s and reduced scattering coefficient, μ_s' at different wavelength for untreated and treated raw milk with

Ethylenediaminetetraacetic acid, (EDTA) based on integrating sphere measurements. The scattering properties and refractive index are dependent on the size of milk fat droplet, where, a high fat globule yields a high refractive index as well as scattering properties (Stocker et al., 2017). Thus, numerical modeling based on Monte Carlo can support the experimental results where the light propagation in milk is analysed theoretically in terms of photon count and photon loss.

3.4 Conclusion

The milk fat analysis in skimmed and full milk was conducted using both experimental and theoretical approaches. All experimental measurements were done using NIR, VIS and FTIR spectrometers. Full milk sample shows the highest absorbance due to high fat globule in full milk compared to others. A large particle of fat globule inside the medium can provide high absorbance at certain wavelength which specifies chemical properties of water and unsaturated fatty acid. The numerical modeling based on Monte Carlo shows that full milk has high photon loss and forward photon count than skimmed milk. The higher internal coefficient in skimmed milk results in higher photon count and photon loss. Full milk sample has higher photon count and photon loss due to higher absorption and scattering in the sample. This study can be used to assist the authority to monitor milk adulteration issue to ensure good human well-being.